REMARKS

Amendments

Claim 1 is amended to delete a grammatically unnecessary comma; Claim 11 is amended to correct numerical dependency; new claim 14 limits the recited fusion protein to particular input and output domains (Specification, p.21, lines 4-5; p.22, line 5; p. 23, line 23 – p.24, line 1; p.10, line 10); new claims 15-22 recite the same methods as claims 10-11 as applied to dependent claims. These amendments introduce no new matter.

35USC112, second paragraph

Claim 1 requires that the input domains interact with each other to (i) allosterically and (ii) external ligand-dependently regulate the output domain. The alternative proposed construction would require replacing "external" with "externally", which is not our meaning.

35USC112, first paragraph (enablement)

The test for enablement is whether the specification enables one skilled in the art to practice the invention as claimed without undue experimentation. Here, the claimed invention is an autoregulated fusion protein comprising an output domain and a plurality of input domains, wherein at least one of the input domains is heterologous to the output domain, and the input domains interact with each other to allosterically and external ligand-dependently regulate the output domain. The fusion proteins link protein input and output domains that are normally not related to provide protein signaling switches analogous to logic gates with diverse and novel input/output properties.

Accordingly, the selected input and output domains are discretionary to the user according to intended use, and essentially any output domain providing a desired activity or binding affinity may be employed, so long as output activity can be regulated by ligand-dependent interaction of the input domains (e.g. Specification, p. 6, lines 13-15). Similarly the selection of input domains is user discretionary, so long as the selected domains interact to provide the requisite ligand-dependent gating of the output domain (e.g. Specification, p.7, lines 18-19).

For example, output domain functional compatibility with the fusion proteins is readily confirmed in routine activity screens (e.g. Specification, p.6, lines 16-17). A wide variety of

output activities may be obtained, depending on the ultimate user application, including catalytic, label-generative, metabolic, apoptotic, and specific-binding output activities (Specification, p.6, lines 17-19). Table 1 lists exemplary output domains shown to have regulatable output activities, including well-studied kinase, phosphatase and protease domains (Specification, p.6, line 25 – p.7, line 7).

Similarly, input domain functional compatibility (demonstrating gating behavior) with the fusion proteins is readily confirmed in routine activity screens. A wide variety of interacting input domains may be used, depending on the ultimate user application, including peptide hormones and cognate receptor ligand binding domains (LBD), immune receptors and cognate antigenic peptides, src-homology domains and cognate peptide ligands, and various catalytic input domains, including modular proteases and both cleavable and non-cleavable pseudosubstrate peptides, modular kinases and peptide substrates, modular phosphatases and phospho-peptide substrates, etc. The input domain interaction can be provided by homo- or hetero-dimerization, by specific pair binding, by higher order complex formation, by enzyme-substrate catalysis (e.g. phosphorylation, glycosylation, prenylation, acylation, lipid modification, etc.). Specification, p.7, lines 18-30.

Preferred input domains comprise native, modular interacting domains which mediate binding of naturally interacting proteins, or natural, modular receptors or enzymes and their cognate ligands and substrates. A wide variety of such modular interacting components has been identified, categorized and subject to grafting. In addition, suitable input domains may be derived from vast public databases of known interacting proteins, including Database of Interacting Proteins (DIP), Database of Ligand-Receptor Proteins, Java-based DIP, and LiveDIP; see, e.g. Xenarios, et al. (2002) NAR 30:303-5; Xenarios, et al.(2001) NAR 29:239-41; Xenarios et al., (2000) NAR 28:289-91; Deane et al.(2002) Mol Cell Prot 1:349-356; Graeber et al. (2001) Nat.Genet. 29:295-300; Marcotte et al. (2001) Bioinformatics 17:359-63; Salwinski et al. (2003) Mol Cell Proteomics. 2002 May;1(5):349-56; Xenarios et al. (2001) Curr Opin Biotechnol 12:334-339. In addition, many protein interaction domains can be mutated to provide alternative specificity binding partners. For example, mutation of a threonine residue of the Src SH2 domain to tryptophan converts ligand-binding specificity from the Src-like pTyr-Glu-Glu-Ile (SEQ ID NO:1), to the signature Grb2 binding motif pTyr-X-Asn (Kimber et al. Molecular

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Cell 2000. 5, 1043-1049). Table 2 lists exemplary input domain binding pairs shown to have external ligand regulatable binding. Specification, p. 8, line 5 – p.18, line 22.

To promote their interactions, one or more of the input domains may be coupled to the fusion protein through a linker or spacer peptide. Linker peptides are widely used in fusion proteins. Linker sequence and length are user-discretionary, though the linkers should not interfere with the output domain when the switch is in the active state (e.g. de-repressed), which is readily confirmed empirically. Preferred linkers often provide structural flexibility and mobility to the input domain. Exemplary use of linker peptides is provided in the disclosed examples of exemplary fusion proteins. Specification, p.7, lines 31 – p.8, line 4.

The claimed protein switches are readily designed or screened such that external ligand activation up-regulates, down-regulates, or otherwise alters output activity. For example, activation can increase, decrease or alter label expression, binding or substrate affinity or specificity, etc. In particular embodiments, the output domain is constitutively active or functional, and in the absence of the ligand, the input domains interact to inhibit the output domain. Where the selected output domain also comprises a suitable input or interaction domain, this endogenous interaction domain may be exploited to create novel allostery in conjunction with a heterologous input or interaction domain. Typically, such endogenous input domains are positioned on the output domain so as to not interfere with the output activity, e.g. the output activity when the fusion protein is de-repressed with ligand. Specification, p.7, lines 8-17.

A wide variety of external ligands may be used to activate the switches by interacting with one or more of the input domains. The external ligands may activate reversibly, such as by reversible competitive or allosteric interaction with one or more of the input domains, or may activate irreversibly, such as through covalent modification. For example, in the case of an SH3 input domain, proline rich peptides can be used as both a second, integral input domain, and as an external competitive ligand. Alternatively, the external ligand can comprise a kinase activity which phosphorylates (covalently modifying) the SH3 domain proximate to the proline-rich binding site, and thereby disrupts interaction of the input domains. Specification, p.18, line 24 – p.19, line 2.

In particular embodiments, the fusion proteins comprise two input domains, both heterologous to the output domain, and which form a specific binding pair. In these

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embodiments, the input domains may also be referred to as receptor-ligand pairs, wherein this internal ligand is one of the input domains, as opposed to the actuating, external ligand which competitively or allosterically disrupts pair-specific binding of the input domains. This input domain binding pair motif may be expanded with additional input domains to provide any desired form of cooperative or antagonistic regulation. For example, the fusion protein may comprise two or more specific binding pairs of input domains which provide higher-order cooperative gating behavior. Accordingly, depending on design or selection, multiple input domains can cooperatively regulate the fusion protein in a wide variety of functionalities, including as an OR-gate, an AND-gate, and an AND-NOT-gate. Similarly, a plurality of output domains can be combined in a single fusion protein, to provide more complex switching. Table 3 provides the compositions of exemplary fusion protein switches, including their corresponding output domain, input domains and regulating external ligand. Specification, p.19, lines 3-24.

The Specification plainly enables one skilled in the art to make and use without undue experimentation an autoregulated fusion protein comprising an output domain and a plurality of input domains, wherein at least one of the input domains is heterologous to the output domain, and the input domains interact with each other to allosterically and external ligand-dependently regulate the output domain. The Specification plainly enables one skilled in the art to make and use such fusion proteins with a wide range of alternative output and input domains. Swapping alternative input and output domains in the recited fusion proteins involves only routine gene splicing and activity screening. A finding of undue experimentation requires much, much more: "the mere fact that the experimentation may have been difficult and time consuming does not mandate a conclusion that such experimentation would have been considered to be 'undue' in this art. Indeed, great expenditures of time and effort were ordinary in the field of vaccine preparation." *Falkner et al. v. Inglis et al.* (Fed.Cir. 05-1324; decided May 26, 2006; favorably quoting underlying Board Decision). Hence, we can not acquiesce in the Action's proposal to eviscerate our invention by restricting the claims to but a single exemplified fusion protein having an N-WASP output domain and SH3 and PDZ input domains" (Action p.4, lines 3-4).

Those skilled in the art recognize that Applicants teachings enable or "pave the way" for creating alternative signal-response elements by protein design:

> In an intriguing variation on this theme, Mark Ptashne nominated two articles from the same group, in which proteins were engineered to mediate novel cellular responses to a particular input. In one article from this group, by Park et
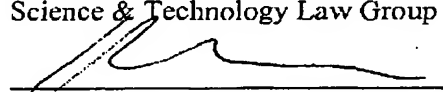
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al. (3), a yeast scaffolding protein was engineered to bind a novel combination of kinases, so that the pheromone α-factor, instead of inducing a mating response, initiated a response normally produced by exposure of cells to high osmolarity. In the second article, by Dueber et al. (4), variants of the actin-regulatory protein N-WASP (neuronal Wiskott-Aldrich signaling protein) were engineered so that they could be activated by a synthetic switch designed by the authors. In nominating this pair of papers, Ptashne noted, "These two remarkable papers show us how, in two quite disparate cases, seemingly intricate and precisely defined protein-protein interactions can be replaced by simpler heterologous interactions without loss of function. These findings shed light on how these systems might have evolved and pave the way for creating new signal-response elements by protein design."

Adler et al. Signaling Breakthroughs of the Year. Adler, Gough, and Ray (2004) Science's STKE 2004: eg1-1 (attached)

Though the Action does not establish a prima facie case, for good measure we provide herewith an expert declaration averring to the foregoing, confirming that the Specification enables one skilled in the art to practice the claimed invention without undue experimentation.

The Examiner is invited to call the undersigned with any suggestions for amending the claims or further clarifying any of the foregoing. Please charge any necessary fees or time extensions relating to this communication to our Dep. Acct. No.19-0750 (order UCSF03-114).

Respectfully submitted,
Science & Technology Law Group

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Encl.   Adler et al. (2004) Science's STKE 2004: eg1-1
        Declaration under 37CFR1.132

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2003: Signaling Breakthroughs of the Year

Elizabeth M. Adler,[1*] Nancy R. Gough,[2] and L. Bryan Ray[3]

(Published 6 January 2004)

Science's STKE rang in 2003 with a new feature on the most notable advances in cell signaling of the past year. The reader response to *2002: Signaling Breakthroughs of the Year* was so overwhelmingly positive that the editors at STKE have decided to make this an annual feature. Thus, we welcome you to 2004 with this article on recent highlights in signaling research. Seven signaling experts were kind enough to share their opinions on what constituted the most exciting research in the area in 2003, paying particular attention to papers likely to blaze the way to new directions in signaling research. This year's participants are Tony Hunter (The Salk Institute, U.S.A.), Ravi Iyengar (Mt. Sinai School of Medicine, U.S.A.), Andre Levchenko (Johns Hopkins University, U.S.A.), Richard Losick (Harvard University, U.S.A.), Mark Ptashne (Memorial Sloan-Kettering Cancer Center, U.S.A.), Eric Vivier (Centre d'Immunologie de Marseille-Luminy, France), and Michael Yaffe (Massachusetts Institute of Technology, U.S.A.). Whereas last year's article emphasized signaling events at the cell membrane, the major themes to emerge this year were structural and organizational, with several experts nominating research concerning the importance of the spatiotemporal organization of cell signaling proteins, and of the role of protein:protein interaction domains in mediating subcellular targeting and determining the response to a given signal.

Eric Vivier noted the importance of "the recent emphasis on the differential utilization of signaling adaptor molecules by a given receptor, or family of receptors" in the context of signaling involving the innate immune response. In particular, Vivier nominated research by Yamamoto *et al.* (*1*) on the Toll-like receptors, which are involved in the recognition of pathogens, and by Diefenbach *et al.* (*2*) on alternately spliced forms of NKG2D, an activating receptor for stress-induced ligands. These papers demonstrate that the identities of the particular adaptors with which a given receptor can associate are crucial to its effector function. In an intriguing variation on this theme, Mark Ptashne nominated two articles from the same group, in which proteins were engineered to mediate novel cellular responses to a particular input. In one article from this group, by Park *et al.* (*3*), a yeast scaffolding protein was engineered to bind a novel combination of kinases, so that the pheromone α-factor, instead of inducing a mating response, initiated a response normally produced by exposure of cells to high osmolarity. In the second article, by Dueber *et al.* (*4*), variants of the actin-regulatory protein N-WASP (neuronal Wiskott-Aldrich signaling protein) were engineered so that they could be activat-

ed by a synthetic switch designed by the authors. In nominating this pair of papers, Ptashne noted, "These two remarkable papers show us how, in two quite disparate cases, seemingly intricate and precisely defined protein-protein interactions can be replaced by simpler heterologous interactions without loss of function. These findings shed light on how these systems might have evolved and pave the way for creating new signal-response elements by protein design."

The theme of protein:protein interaction domains, as well as that of the critical importance of spatiotemporal organization to cell signaling, was also raised by Tony Hunter, who nominated the identification of the polo box (*5-7*) and BRCT domains (*8-10*) as novel phosphopeptide binding motifs "as an important advance in understanding signaling by serine/threonine protein kinases." The BRCT domain is a protein-protein interaction motif found in many proteins involved in the response to DNA damage, including BRCA1, a tumor suppressor protein, mutant forms of which are associated with breast and ovarian cancer. BRCT repeats can reportedly interact with sites phosphorylated by DNA damage-activated kinases, such as ATM (ataxia telangiectasia mutated), potentially allowing recruitment of BRCT repeat-containing proteins to sites of DNA damage and repair. The polo box domain, a noncatalytic motif unique to the polo-like kinases, a family of mitotic and checkpoint kinases, allows polo-like kinases to be recruited to appropriate substrates and cellular structures at particular stages of the cell cycle. The implication of both the BRCT and polo box domains in cell cycle- and phosphorylation-dependent protein targeting adds new weight to the emerging role of posttranslational modification in regulating the formation of signaling networks, and provides potential new targets for therapeutic intervention.

Richard Losick nominated research by Shan and Walter (*11*) concerning the mechanisms whereby two bacterial guanosine triphosphatases (GTPases) Ffh and FtsY, which function as a subunit of the bacterial signal recognition particle (SRP) and SRP receptor, respectively—regulate the targeting of secreted proteins to the plasma membrane. These two GTPases, which do not require guanine nucleotide exchange factors, reciprocally activate one another. Shan and Walter showed that the interaction of the two proteins caused a conformational change in FtsY that induced nucleotide-binding specificity. Noting that this was "only the first chapter" in "one of the loveliest stories I have heard in the signal transduction field," Losick offered to keep us apprised as new developments concerning these very unusual GTPases continue to unfold.

Ravi Iyengar nominated two articles indicating that Rap (a GTPase implicated in integrin signaling and known to function as a Ras antagonist) may play a key role in integrating different signaling pathways and in coordinating various physiological responses at the synapse. In the first article, by Morozov *et al.* (*12*), Rap was shown to couple signaling through the adenosine 3',5'-monophosphate (cAMP) pathway to the regulation of a pool of p42/44 mitogen associated protein kinase (MAPK) and to play a role in mediating both short- and long-term events in-

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volved in the induction of long-term potentiation of synaptic efficacy. In the second article, by Pak and Sheng (*13*), Rap regulation was implicated in the activity-dependent modulation of synaptic structure. Noting that these two studies enhance our "understanding of the role of Rap in the spatiotemporal integration of signals," Iyengar suggested that regulation of Rap activity could thus "integrate synaptic function at multiple levels."

Noting that "although we believe that signal transduction is somewhat stereotypical, with signals proceeding more or less linearly...an ever increasing body of evidence suggests interesting positive and negative feedback possibilities," Andre Levchenko nominated research from two groups, Lorenz *et al.* (*14*) and Corbit *et al.* (*15*), that uncovered an unexpected form of positive feedback. Phosphorylation of Raf kinase inhibitor protein (RKIP) following adrenergic stimulation and activation of protein kinase C causes RKIP to shift from inhibiting Raf-1 to inhibiting G protein-coupled receptor kinase 2 (GRK-2). Both the relief of Raf-1 inhibition and the inhibition of GRK-2 lead to enhanced signaling through the activating receptor and thus represent a novel form of dual positive feedback mediated through phosphorylation of a single protein.

Finally, Michael Yaffe, whose own work on the identification of the BRCT and polo box domains as phosphoserine/threonine binding motifs figured prominently in the foregoing nominations, contributed three nominations concerning research taking place over the last few years. The first was for major advances in understanding TOR (target of rapamycin) signaling. TOR, which plays a key role in regulating cell growth and proliferation, is inhibited by rapamycin, an antifungal agent that is used clinically as an immunosuppressive agent and in cancer therapy. Yaffe cited recent findings identifying TOR-interacting proteins and the link between TOR and phosphoinositide 3-kinase (PI3K) signaling pathways, that "begin to explain how TOR controls cell growth and size, how rapamycin acts, and how insulin might function to control cell growth through the PI3K pathway" (*16-21*). The second nomination concerned the role of phosphorylation-mediated ubiquitin degradation in regulation of the cell cycle, in particular the requirement for multisite phosphorylation of the cyclin dependent kinase inhibitor Sic1 for degradation and thus the transition from G1 to S phase (*22-24*). Finally, Yaffe predicted that the "very recent idea that a persistent DNA damage signal might underlie the mechanism of telomere shortening-initiated senescence" was an important advance that was likely to gain momentum over the next few years (*25-26*).

The STKE editors also put their heads together and had a few suggestions of breaking research. The tumor suppressor p53, which is well known for its nuclear functions in mediating responses to DNA damage, is beginning to be recognized for several nonnuclear activities or functions that extend beyond responding to cellular stress. For example, some of the apoptotic activity of p53 may be occurring through interactions with the mitochondria (*27*). A role for p53 as a Smad partner in signaling by transforming growth factor β also expands the functions of p53 to include regulation of embryogenesis (*28*). As with many proteins, the functions first identified for p53 are only the beginning of multiple diverse activities waiting to be uncovered. Also notable was the accumulation of evidence of a key role for the endoplasmic reticulum in signals causing apoptosis. Although release of factors from the mitochondria has been central to the cell death pathway, a series of articles suggest that the ER and mitochondria actually cooperate to produce a positive feedback system that results in apoptosis (*29-33*)

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* Getting SET for cell death. *Sci. STKE* 2003, tw112 (2003).

References

1. M. Yamamoto, S. Sato, H. Hemmi, S. Uematsu, K. Hoshino, T. Kaisho, O. Takeuchi, K. Takeda, S. Akira, TRAM is specifically involved in the Toll-like receptor 4–mediated MyD88-independent signaling pathway. *Nat. Immunol.* 4, 1144-1150 (2003).
2. A. Diefenbach, E. Tomasello, M. Lucas, A. M. Jamieson, J. K. Hsia, E. Vivier, D. H. Raulet, Selective associations with signaling proteins determine

EDITORIAL GUIDE

stimulatory versus costimulatory activity of NKG2D. *Nat. Immunol.* **3**, 1142-1149 (2002).

3. S.-H. Park, A. Zarrinpar, W. A. Lim, Rewiring MAP kinase pathways using alternative scaffold assembly mechanisms. *Science* **299**, 1061-1064 (2003).

4. J. E. Dueber, B. J. Yeh, K. Chak, W. A. Lim, Reprogramming control of an allosteric signaling switch through modular recombination. *Science* **301**, 1904-1907 (2003).

5. I. A. Manke, D. M. Lowery, M. B. Yaffe, BRCT repeats as phosphopeptide-binding modules involved in protein targeting. *Science* **302**, 636-639 (2003).

6. X. Yu, C. Christiano, S. Chini, M. He, G. Mer, J. Chen, The BRCT domain is a phospho-protein binding domain. *Science* **302**, 639-642 (2003).

7. M. Rodriguez, X. Yu, J. Chen, Z. Songyang, Phosphopeptide binding specificities of BRCA1 COOH-terminal (BRCT) domains. *J. Biol. Chem.* **278**, 52914-52918 (2003).

8. A. E. H. Elia, L. C. Cantley, M. B. Yaffe, Proteomic screen finds pSer/pThr-binding domain localizing Plk1 to mitotic substrates. *Science* **299**, 1228-1231 (2003).

9. A. E. Elia, P. Rellos, L. F. Haire, J. W. Chao, F. J. Ivin, K. Hoepker, D. Mohammad, L. C. Cantley, S. J. Smerdon, M. B. Yaffe, The molecular basis for phosphodependent substrate targeting and regulation of Plks by the Polo-box domain. *Cell* **115**, 83-95 (2003).

10. K. Y. Cheng, E. D. Lowe, J. Sinclair, E. A. Nigg, L. N. Johnson, The crystal structure of the human polo-like kinase-1 polo box domain and its phospho-peptide complex. *EMBO J.* **22**, 5757-5768 (2003).

11. S. Shan, P. Walter, Induced nucleotide specificity in a GTPase. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 4480-4485 (2003).

12. A. Morozov, I. A. Muzzio, R. Bourtchouladze, N. Van-Strien, K. Lapidus, D. Q.Yin, D. G. Winder, J. P. Adams, J. D. Sweatt, E. R. Kandel, Rap1 couples cAMP signaling to a distinct pool of p42/44MAPK regulating excitability, synaptic plasticity, learning, and memory. *Neuron* **39**, 309-325 (2003).

13. D. T. Pak, M. Sheng, Targeted protein degradation and synapse remodeling by an inducible protein kinase. *Science* **302**, 1368-1373 (2003).

14. K. Lorenz, M. J. Lohse, U. Quitterer, Protein kinase C switches the Raf kinase inhibitor from Raf-1 to GRK-2. *Nature* **426**, 574-579 (2003).

15. K. C. Corbit, N.Trakul, E. M. Eves, B. Diaz, M. Marshall, M. R. Rosner, Activation of Raf-1 signaling by protein kinase C through a mechanism involving Raf kinase inhibitory protein. *J. Biol. Chem.* **278**, 13061–13068 (2003).

16. D.-H. Kim, D. D. Sarbassov, S. M. Ali, J. E. King, R. R. Latek, H. Erdjument-Bromage, P. Tempst, D. M. Sabatini, mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell* **110**, 163-175 (2002).

17. K. Hara, Y. Maruki, X. Long, K. Yoshino, N. Oshiro, T. Hidayat, C. Tokunaga, J. Avruch, K. Yonezawa, Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action. *Cell* **110**, 177-189 (2002).

18. B. D. Manning, A. R. Tee, M. N. Logsdon, J. Blenis, L. C. Cantley, Identification of tuberous sclerosis complex-2 tumor suppressor gene product tuberin as a target of the phosphoinositide 3-kinase/Akt pathway. *Mol. Cell* **10**, 151-162 (2002).

19. K. Inoki, Y. Li, T. Xu, K. L. Guan, Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. *Genes Dev.* **17**, 1829-1834 (2003).

20. A. R. Tee, B. D. Manning, P. P. Roux, L. C. Cantley, J. Blenis, Tuberous sclerosis complex gene products, tuberin and hamartin, control mTOR signaling by acting as a GTPase-activating protein complex toward Rheb. *Curr. Biol.* **13**, 1259-1268 (2003).

21. K. Inoki, T. Zhu, K. L. Guan, TISC2 mediates cellular energy response to control cell growth and survival. *Cell* **115**, 577-590 (2003).

22. P. Nash, X. Tang, S. Orlicky, Q. Chen, F. B. Gertler, M. D. Mendenhall, F. Sicheri, T. Pawson, M. Tyers, Multisite phosphorylation of a CDK inhibitor sets a threshold for the onset of DNA replication. *Nature* **414**, 514-521 (2001).

23. S. Orlicky, X. Tang, A. Willems, M. Tyers, F. Sicheri, Structural basis for phosphodependent substrate selection and orientation by the SCFCdc4 ubiquitin ligase. *Cell* **112**, 243-256 (2003).

24. P. Klein, T. Pawson, M. Tyers, Mathematical modeling suggests cooperative interactions between a disordered polyvalent ligand and a single receptor site. *Curr. Biol.* **13**, 1669-1678 (2003).

25. H. Takai, A. Smogorzewska, T. de Lange, DNA damage foci at dysfunctional telomeres. *Curr. Biol.* **13**, 1549-1556 (2003).

26. F. d'Adda di Fagagna, P. M. Reaper, L. Clay-Farrace, H. Fiegler, P. Carr, T. Von Zglinicki, G. Saretzki, N. P. Carter, S. P. Jackson, A DNA damage checkpoint response in telomere-initiated senescence. *Nature* **426**, 194-198 (2003).

27. M. Mihara, S. Erster, A. Zaika, O. Petrenko, T. Chittenden, P. Pancoska, U. M. Moll, p53 has a direct apoptogenic role at the mitochondria. *Mol. Cell* **11**, 577-590 (2003).

28. M. Cordenonsi, S. Dupont, S. Maretto, A. Insinga, C. Imbriano, S. Piccolo, Links between tumor suppressors: p53 is required for TGF-β gene responses by cooperating with Smads. *Cell* **113**, 310-314 (2003).

29. L. Scorrano, S. A. Oakes, J. T. Opferman, E. H. Cheng, M. D. Sorcinelli, T. Pozzan, S. J. Korsmeyer, BAX and BAK regulation of endoplasmic reticulum Ca^{2+}: A control point for apoptosis. *Science* **300**, 135-139 (2003).

30. C. Hetz, M. Russelakis-Carneiro, K. Maundrell, J. Castilla, C. Soto, Caspase-12 and endoplasmic reticulum stress mediate neurotoxicity of pathological prion protein. *EMBO J.* **22**, 5435-5445 (2003).

31. M. P. Mattson, S. L. Chan, Calcium orchestrates apoptosis. *Nat. Cell Biol.* **5**, 1041-1043 (2003).

32. R. Hegde, S. M. Srinivasula, P. Datta, M. Madesh, R. Wassell, Z. Zhang, N. Cheong, J. Nejmeh, T. Fernandes-Alnemri, S. Hoshino, E. S. Alnemri, The polypeptide chain-releasing factor GSPT1/eRF3 is proteolytically processed into an IAP-binding protein. *J. Biol. Chem.* **278**, 38699-38706 (2003).

33. Z. Fan, P. J. Beresford, D. Y. Oh, D. Zhang, J. Lieberman, Tumor suppressor NM23-H1 is a granzyme A-activated DNase during CTL-mediated apoptosis, and the nucleosome assembly protein SET is its inhibitor. *Cell* **112**, 659-672 (2003).